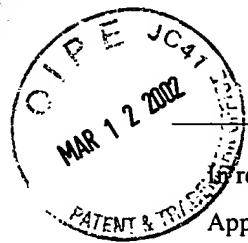


1642

PATENT
TECH CENTER 1600/2900
MAR 15 2002

Case Docket No. GENENT.333CPC4C
Date: March 6, 2002

RECEIVED



Re application of : PRESTA et al.
 App. No. : 09/966,147
 Filed : September 27, 2001
 For : HUMAN TRK
 RECEPTORS AND
 NEUROTROPHIC
 FACTOR INHIBITORS
 Examiner : Not yet assigned
 Art Unit : 1642

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first class mail in an envelope addressed to: United States Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202, on

March 6, 2002

(Date)

Ginger R. Dreger, Reg. No. 33,055

UNITED STATES PATENT AND TRADEMARK OFFICE
 P.O. Box 2327
 Arlington, VA 22202

Sir:

Transmitted herewith are the following.

- (X) Preliminary Communication.
- (X) Declaration Under 37 C.F.R. § 1.132 with accompanying Figs. A-F.
- (X) Return prepaid postcard.
- (X) The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 11-1410.

Chas R. Dreger
 Ginger R. Dreger
 Registration No. 33,055
 Attorney of Record



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Presta <i>et al.</i>)	Group Art Unit: 1642
Appl. No.	:	09/966,147)	
Filed	:	September 27, 2001)	
For	:	HUMAN TRK RECEPTORS AND NEUROTROPHIC FACTOR INHIBITORS)	
Examiner	:	not yet assigned)	

PRELIMINARY COMMUNICATION

UNITED STATES PATENT AND TRADEMARK OFFICE
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

Attached to the present Communication is a Declaration (and accompanying Figures A-F) under 37 C.F.R. § 1.132. Applicants respectfully request that this Declaration be entered into the record of the above cited Application prior to examination of the Application on the merits.

Please charge any fees or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,
KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: March 6, 2002

By: Ginger Dreger
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(415) 954-4114

#6

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O P E R A T I O N S
M A R 1 2 2002
P A T E N T & T R A D E M A R K S
J C A T 2002

Applicants	:	Presta <i>et al.</i>)
Appl. No.	:	09/966,147)
Filed	:	September 27, 2001)
For	:	HUMAN trk RECEPTORS AND NEUROTROPHIC FACTOR INHIBITORS)
Examiner	:	not yet assigned)

DECLARATION UNDER 37 C.F.R. § 1.132

UNITED STATES PATENT AND TRADEMARK OFFICE
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

I, Jo-Anne Hongo, do hereby declare and say as follows:

1. I am a Senior Research Associate in the Antibody Technology Section of the Department of Immunology at Genentech, Inc., in South San Francisco, California.
2. The ability of anti-Trk antibodies to block receptor/ligand interaction and regulate receptor activity of TrkA, TrkB and TrkC neurotrophin receptors was tested by me and others using protocols known in the art. I have read and understand all experiments described in this Declaration. The two protocols used herein were a ELISA blocking assay and a kinase receptor activation (KIRA) assay. As used herein, the KIRA assay is used quantitatively to measure receptor protein tyrosine kinase (rPTK) activation and receptor tyrosine autophosphorylation *in vitro* in response to a particular ligand or test compound (as discussed in the Specification of the above-identified application, at pages 66-68). A variation of this assay is used herein to identify

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Filed : September 27, 2001

antagonists of a rPTK. The results of these assays are shown in Figures A-F, which are appended to and form part of the present Declaration.

3. In one experiment, the ability of anti-Trk antibodies to block NGF interaction with the TrkA receptor was tested using a blocking ELISA. In this experiment, ELISA plate wells were coated with human NGF at a concentration of 1 μ g/mL. To these wells was added a human TrkA-IgG fusion protein (50 ng/mL), where the human TrkA open reading frame was fused to the human immunoglobulin IgG Fc domain, preincubated with or without various anti-TrkA or control monoclonal antibodies at a concentration of 5 μ g/mL. After incubation, the wells were washed, and the amount of TrkA-IgG fusion protein remaining in the wells (and presumably bound to the immobilized hNGF) was detected using a goat anti-human IgG Fc antibody that is conjugated to horse radish peroxidase (HRP). After incubation with the HRP conjugate, the wells were washed again, and the bound TrkA-IgG remaining in the well was quantitated by addition of the chromogenic HRP substrate o-phenylenediamine (OPD), which yields a reaction product that is quantitated at 490 nm wavelength. The results of this experiment are shown in Figure A. In this experiment, five anti-TrkA antibodies were tested, as well as one control antibody. As can be seen in Figure A, the 1488, 1502 and 1503 antibodies show a strong ability to block the physical interaction of TrkA with its cognate ligand NGF compared to a control antibody.

4. In another set of experiments, monoclonal antibodies specific for TrkA or TrkC polypeptides were tested for antagonistic activity on TrkA and TrkC receptor signalling, respectively, using the KIRA assay. Activation of Trk receptor signalling by neurotrophin ligands results in a tyrosine autophosphorylation event in the receptors. Thus, the presence of phosphorylated tyrosine residues on the intracellular domain of the Trk receptor is indicative of receptor activation. These Trk phosphotyrosine positions are detectable using an ELISA protocol with a suitable capture antibody and an anti-phosphotyrosine antibody. The ability of a particular anti-Trk monoclonal antibody to inhibit receptor signalling activity is demonstrated by reduced levels of phosphotyrosine on the Trk receptor in response to activation by a neurotrophin ligand (e.g., NGF or NT-3).

5. Briefly, the following KIRA protocol was used.

- A. Two stably transfected chinese hamster ovary (CHO) cell lines were constructed which overexpressed chimeric forms of the TrkA or TrkC receptors. The chimeric proteins were formed by fusing the TrkA and TrkC receptors with a 27 amino acid peptide derived from the Herpes Simplex Virus glycoprotein D (gD) tag. This tag facilitates immobilization of the extracted receptor protein in the subsequent ELISA.
- B. Approximately 10^5 CHO cells expressing either TrkA-gD or TrkC-gD receptor were plated into the wells of 96-well culture dishes. Following establishment of the cultures, anti-TrkA or anti-TrkC monoclonal antibodies were added to the cultures at concentrations from 0.130 to 100 μ g/mL and allowed to incubate for 30 minutes.
- C. Following this incubation, the cells were stimulated by the addition of 150-250 pM human NGF (when using the TrkA-gD expressing cells) or NT-3 (when using the TrkC-gD expressing cells) to the wells and incubated for an additional 25 minutes.
- D. Following the treatment, the cultures were lysed in 100 μ L solubilization buffer (150 mM NaCl, 0.5% Triton-X 100, 50 mM HEPES, pH 7.5, Na₃VO₄ [phosphatase inhibitor], Bacitracin, and PMSF, aprotinin, and AEBSF [protease inhibitors]) for 60 minutes, and the cell lysates were collected.
- E. The cell lysates were then analyzed using an ELISA protocol to quantitate the amount of phosphotyrosine on the recombinant Trk-gD receptors, as a measure of receptor activation. To accomplish this, 85 μ L of the cell lysates were added to ELISA plate wells that had been previously coated with an anti-gD peptide monoclonal capture antibody, and allowed to incubate for 2 hours.
- F. The wells were then washed, and biotinylated anti-phosphotyrosine antibody was added to the wells, and incubated for two hours.
- G. Following the incubation, the wells were washed, and strepavidin-HRP conjugate was added to each well, and incubated for 30 minutes.
- H. The wells were washed again, then exposed to the chromogenic HRP substrate 3,3',5,5'-tetramethyl-benzidine (TMB). The color development reactions were allowed to proceed for 10 minutes, then were stopped using 1 M H₃PO₄. Color development was quantitated by colorimetric determination at the dual wavelength 450/650 nm, where Abs_{450nm} is a

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measure of the chromogenic TMB product and $\text{Abs}_{650\text{nm}}$ is a reference measure that monitors progression of the chromogenic reaction.

6. Figure B shows the results of a KIRA assay testing anti-TrkA monoclonal antibodies for the ability to suppress TrkA-gD receptor activation (*i.e.*, TrkA receptor autophosphorylation). Four anti-TrkA monoclonal antibodies were tested (1486, 1488, 1502 and 1503). Relative concentrations of the antibodies are shown on the X-axis, and detection of phosphotyrosine residues is shown on the Y-axis. The graph depicts the ELISA quantitation of phosphotyrosine in the TrkA-gD receptor following stimulation with NGF in the presence of the specific antibodies at various antibody concentrations. As can be seen in this graph, all four antibodies demonstrate some ability to suppress the tyrosine autophosphorylation of the TrkA receptor in a dose-dependent manner.

7. Figure C shows the Abs_{450} readings of a KIRA assay using TrkC-gD expressing cells in the presence of five different anti-TrkC monoclonal antibodies. The antibodies were used over a 1,000-fold range of concentrations (0.13 to 100 $\mu\text{g/mL}$). This ELISA quantitation measures the phosphotyrosine in the TrkC-gD receptor following stimulation with NT-3 ligand and in the presence of the specific antibodies. Figure D shows these same results graphically. On this graph, the activity of the TrkC-gD receptor in the presence of NT-3 ligand and a non-specific control monoclonal antibody is shown as a dotted line at the top of the graph, at an Abs_{450} value of 0.600. As can be seen in this graph, each of the specific monoclonal antibodies shows some ability to suppress TrkC-gD tyrosine autophosphorylation.

8. In a similar experiment as above, Figure E shows the Abs_{450} readings of a KIRA assay using TrkC-gD expressing cells and three different anti-TrkC monoclonal antibodies. The antibodies were used over a 1,000-fold range of concentrations (0.13 to 100 $\mu\text{g/mL}$). This ELISA measures TrkC-gD receptor activation following stimulation with NT-3 and in the presence of the specific antibodies. Figure F shows these same results graphically. On this graph, the activity of the TrkC-gD receptor in the presence of NT-3 ligand and a non-specific control monoclonal antibody is shown as a dotted line at the top of the graph, at an Abs_{450} value

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of 0.600. As can be seen in this graph, each of the antibodies can suppresses receptor tyrosine autophosphorylation to some extent.

9. As can be seen from the experiments described in this Declaration, the anti-Trk antibodies provided by the present invention have the ability to block the biological signalling activity of Trk receptors. The inhibitory activity of these antibodies is indicative of their usefulness in therapies for diseases where expression of a neurotrophin or a neurotrophin receptor (*i.e.*, a Trk receptor) correlates with a disease state.

10. Diseases where expression of Trk receptors correlates with the disease pathology are known in the art (see the Specification at page 4, lines 6-10, and page 68, lines 18-29). Based on the results of experiments summarized in this Declaration, it is my considered scientific opinion that molecules that antagonize neurotrophin or Trk activity (*e.g.*, Trk-specific antibodies or Trk immunoadhesins) find use in the treatment of diseases characterized by neurotrophin or Trk receptor expression as determined by mRNA or protein assessment.

11. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. I declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

030102

Date

Jo-Anne Hongo

Jo-Anne Hongo

Figure A

Anti-TrkA Monoclonal Antibody Blocking Activities
as Determined by Competitive ELISA

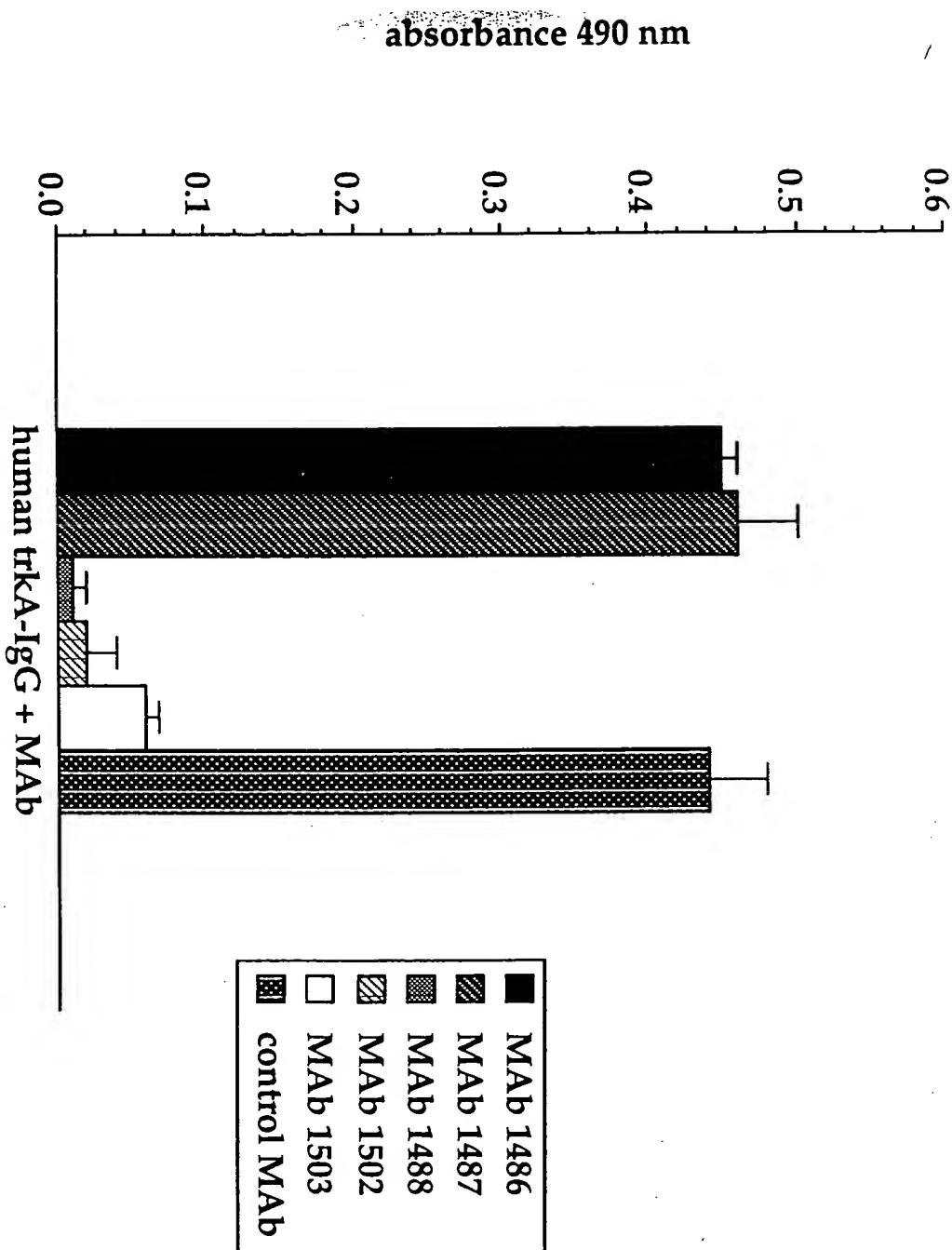


Figure B

TrkA Kinase Receptor Activation Assay (KIRA)
using Anti-TrkA Monoclonal Antibodies

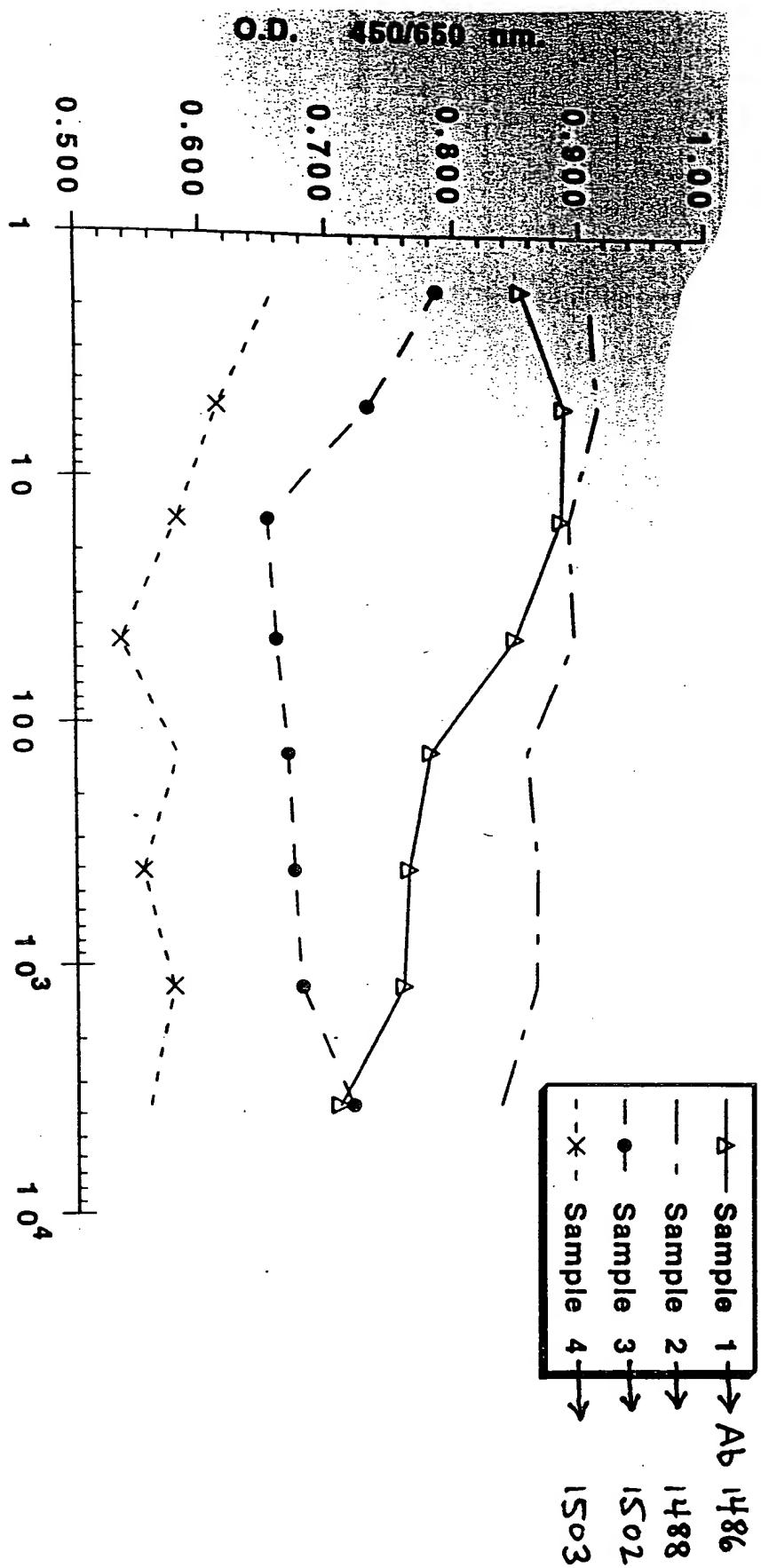


Figure C

**Data from TrkC Kinase Receptor Activation Assay (KIRA)
Using Anti-TrkC Monoclonal Antibodies**

Std Curve	
OD, Ave	sd
0.546	0.023
0.629	0.062
0.541	0.024
0.505	0.053
0.382	0.041
0.261	0.001
0.203	0.006
0.188	0.006

OD, Ave	sd	OD, Ave	sd	OD, Ave	sd	OD, Ave	sd			
#20150-65		#20150-66		#20150-67A		#20150-68				
1.00		0.502	0.174	0.318	0.013	0.343	0.013			
0.445	0.086					0.474	0.125			
33.3	0.439	0.121	0.424	0.105	0.319	0.005	0.351	0.049	0.304	0.018
11.1	0.431	0.064			0.315	0.004	0.320	0.012	0.384	0.055
3.7	0.531	0.069	0.482	0.033	0.349	0.031	0.373	0.064	0.352	0.056
1.2	0.538	0.030	0.406	0.051	0.335	0.005	0.350	0.013	0.363	0.066
0.4	0.388	0.004	0.388	0.081	0.346	0.030	0.357	0.002	0.412	0.021
0.13	0.453	0.071	0.502	0.026	0.543	0.074	0.387	0.011	0.417	0.023
No Ligand	0.274	0.108	0.166	0.003	0.181	0.018	0.252	0.030	0.211	0.015

Figure D

Graph of TrkC Kinase Receptor Activation Assay (KIRA) Data

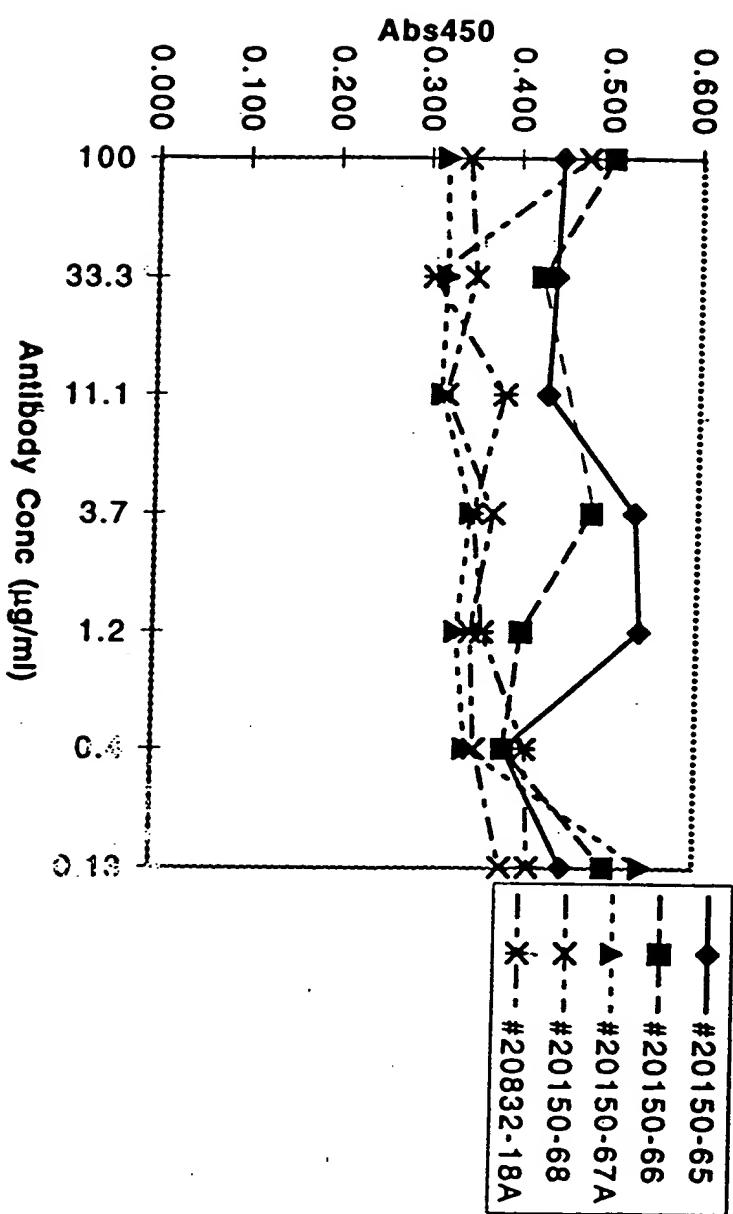


Figure E

Data from TrkC Kinase Receptor Activation Assay (KIRA)
Using Anti-TrkC Monoclonal Antibodies

Std Curve		OD, Ave		sd		OD, Ave		sd		OD, Ave		sd	
OD	Ave	sd	#20832-18B	OD	Ave	sd	#20150-69	OD	Ave	sd	#20150-70		
0.499	0.007		100	0.274	0.004	0.511	0.089	0.263	0.006				
0.507	0.004		33.3	0.257	0.004	0.434	0.013	0.280	0.004				
0.475	0.004		11.1	0.286	0.023	0.463	0.024	0.292	0.004				
0.421	0.002		3.7	0.276	0.001	0.462	0.019	0.293	0.011				
0.317	0.009		1.2	0.303	0.006	0.508	0.051	0.300	0.005				
0.237	0.004		0.4	0.342	0.002	0.474	0.006	0.309	0.008				
0.204	0.009		0.13	0.331	0.004	0.407	0.003	0.317	0.015				
0.170	0.001		No Ligand	0.215	0.021	0.211	0.001	0.185	0.004				

Figure F

Graph of TrkC Kinase Receptor Activation Assay (KIRA) Data

